NEUTRALIZING ANTIBODY ASSAY FOR THERAPEUTIC PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 63/018,821, filed May 1, 2020, U.S. Provisional Patent Application No. 63/041,768, filed Jun. 19, 2020 and U.S. Provisional Patent Application No. 63/172,488 filed Apr. 8, 2021 which are each herein incorporated by reference.

FIELD

[0002] This application relates to assay methods, modules, and kits for conducting diagnostic assays for detection of neutralizing antibodies against therapeutic proteins.

BACKGROUND

[0003] Administration of biological therapeutics to a patient can induce an undesirable immunogenic response in the patient that can lead to the development of anti-drug antibodies (ADAs) (Mire-Sluis, A. R., et al., *J Immunol Methods,* 289(1):1-16 (2004)). Neutralizing antibodies (NAbs) are a subset of ADAs that inhibit binding of the drug to its target, rendering the drug biologically inactive. By definition, NAbs neutralize the effect of the drug, potentially reducing clinical activity. In addition, where the drug is a biological mimic of an endogenous protein, NAbs may cross-react with the drug's endogenous analogue, which can have critical consequences for drug safety (Finco, D., et al., *J Pharm Biomed Anal,* 54(2):351-358 (2011); Hu, J., et al., *J Immunol Methods,* 419:1-8 (2015)).

[0004] Detection of an immunogenic response involves a tiered approach where a sample is first tested for the presence of ADAs, typically using a bridging immunoassay (Mire-Sluis, A. R., et al., *J Immunol Methods,* 289(1):1-16 (2004)). Further characterization of the ADA response may include a titer assay to determine the relative amount of ADAs, and an assay to determine whether the antibody response is neutralizing (Wu, B., et al., *AAPS Journal,* 18(6):1335-1350 (2016); Shankar, G, et al., *J Pharm Biomed Anal* 48(5):1267-1281 (2008); Gupta, S., et al., *J Pharm Biomed Anal,* 55(5):878-888 (2011)).

[0005] NAb assays can be subject to interference that prevents accurate quantitation of neutralization against the therapeutic protein. For example, if the endogenous drug target is soluble, it may be present in the subject sample and competitively bind with the therapeutic, creating a false positive NAb signal. There may also be residual drug in the subject sample from previous administrations of the therapeutic, which can competitively bind to NAbs and create a false negative NAb signal. Different techniques have been developed to deal with these sources of interference to obtain an accurate quantitation of NAbs (Xu, W., et al., *J Immunol Methods,* 462:34-41 (2018); Xu, W., et al., *J Immunol Methods,* 416:94-104 (2015); Xiang, Y., et al., *AAPS Journal,* 21(1):4 (2019); Sloan, J. H., et al., *Bioanalysis,* 8(20):2157-2168 (2016)).

[0006] An additional source of potential interference that has not yet been characterized is interference by a residual drug, different from the therapeutic protein being tested, that competitively binds to the same drug target as the therapeutic, which would create a false positive NAb signal. As such, a strategy to mitigate this type of interference has also not been developed to date.

[0007] Therefore, it will be appreciated that a need exists for methods to identify and mitigate interference from competing drugs in ligand binding assays or cell-based assays for the detection of neutralizing antibodies against therapeutic proteins.

SUMMARY

[0008] This disclosure provides a method for detecting a neutralizing agent to a therapeutic protein in a sample. In some exemplary embodiments, the method comprises (a) contacting said sample having said neutralizing agent and a competing drug to (i) said therapeutic protein, (ii) a target of said therapeutic protein, and (iii) a mitigating agent; (b) measuring a binding of said therapeutic protein to said target; and (c) comparing the result of (b) to a control measurement to detect said neutralizing agent.

[0009] In one aspect, said control measurement is obtained by measuring binding of said therapeutic protein to said target in the absence of a neutralizing agent. In another aspect, said neutralizing agent is a neutralizing antibody.

[0010] In one aspect, said therapeutic protein is an antibody, a soluble receptor, an antibody-drug conjugate, or an enzyme. In a specific aspect, said therapeutic protein is a monoclonal antibody. In yet another specific aspect, said monoclonal antibody is an anti-PD-1 antibody, an anti-TNF antibody, an anti-PD-L1 antibody, an anti-EGFR antibody, an anti-CD20 antibody, an anti-CD38 antibody, or an anti-LAG3 antibody.

[0011] In one aspect, said therapeutic protein is a bispecific antibody. In a specific aspect, said bispecific antibody is a CD20×CD3 antibody, a BCMA×CD3 antibody, a EGFR×CD28 antibody, or a CD38×CD28 antibody.

[0012] In one aspect, said therapeutic protein is immobilized to a solid support. In another aspect, said therapeutic protein is labeled for detection. In a specific aspect, said label is detectable by fluorescence, chemiluminescence, electrochemiluminescence, radioactivity, or affinity purification. In yet another specific aspect, said label comprises ruthenium.

[0013] In one aspect, said target is an antigen, a receptor, a ligand, or an enzymatic substrate. In another aspect, said target is a cell surface protein. In yet another aspect, said target is a recombinant protein. In yet another aspect, said target is expressed by a cell. In a specific aspect, said cell is a HEK293 cell, a MOLP-8 cell, a Jurkat cell, or a modified version thereof.

[0014] In one aspect, said target is immobilized to a solid support. In another aspect, said target is labeled for detection. In a specific aspect, said label is detectable by fluorescence, chemiluminescence, electrochemiluminescence, radioactivity, or affinity purification. In another aspect, said target is an enzymatic substrate. In yet another aspect, said target is CD20, CD3, BCMA, PD-1, EGFR, CD28, CD38, TNF, PD-L1, or LAG3. In yet another aspect, said method additionally comprises a second target.

[0015] In one aspect, said competing drug is a monoclonal antibody. In a specific aspect, said competing drug is rituximab, pembrolizumab, nivolumab, ocrelizumab, obinutuzumab, ofatumumab, ibritumomab tiuxetan, tositumomab, ublituximab, cetuximab, daratumumab, or adalimumab. In another aspect, said competing drug is a bispecific antibody.